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TECHNICAL MANUSCRIPT 524

SEX SPECIFICITY  
OF A PASTEURELLA BACTERIOPHAGE

Dorothy M. Molnar  
William D. Lawton

JUNE 1969

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TECHNICAL MANUSCRIPT 524

SEX SPECIFICITY OF A PASTEURELLA BACTERIOPHAGE

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Medical Bacteriology Division  
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#### ABSTRACT

Phage H, thought to be specific for Pasteurella pestis, was shown to plate efficiently on F<sup>-</sup> strains of Escherichia coli but not on F<sup>+</sup>, F', or Hfr strains. The phage was adsorbed rapidly to F<sup>-</sup> strains but was not adsorbed to strains carrying F. Comparison with seven other reported female-specific phages showed that, although phage H was similar to the other phages in some characteristics, the exceptionally low efficiency of plating ( $<10^{-9}$ ) on F-containing cells makes phage H a particularly useful female-specific phage.

## CONTENTS

Acknowledgments . . . . .	2
Abstract . . . . .	2
I. INTRODUCTION . . . . .	5
II. MATERIALS AND METHODS . . . . .	5
A. Phage . . . . .	5
B. Bacterial Strains . . . . .	5
C. Media and Cultural Conditions . . . . .	6
D. Propagation of Phage . . . . .	6
E. Assay of Phage . . . . .	6
III. RESULTS . . . . .	6
A. Characteristics of Phage H . . . . .	6
B. Host Range of Phage H . . . . .	7
C. Specificity of Phage H for F <sup>-</sup> Strains of <u>E. coli</u> . . . . .	7
D. Adsorption of Phage H . . . . .	9
E. Comparison of Phage H with Other Female-Specific Phages . . . . .	12
F. Evidence that Phage H and T3 are Different . . . . .	12
IV. DISCUSSION . . . . .	15
Literature Cited . . . . .	17
Distribution List . . . . .	19
DD Form 1473 . . . . .	21

## FIGURES

1. Single-Step Growth Curve of Phage H on <u>E. coli</u> AB-6 . . . . .	8
2. Influence of the F Factor in Strains of <u>E. coli</u> on the Lysis of Broth Cultures by Phage H . . . . .	10
3. Adsorption of Phage H to Strains of <u>E. coli</u> of Different Sexual Types . . . . .	11
4. Adsorption of T3 to <u>E. coli</u> B, AB-6, and HfrC . . . . .	14

## TABLES

1. Influence of the F Factor in Strains of <u>E. coli</u> on the Efficiency of Plating of Phage H . . . . .	9
2. Comparison of Phage H with Other Reported Sex-Specific Phages . . . . .	13

3. Differentiation Between Phages H and T3 by Selection of a Host Range  
Mutant of T3 on E. coli AB-6 . . . . . 15

## I. INTRODUCTION\*

During an investigation into the use of phage to eliminate donor cells in matings between Pasteurella pseudotuberculosis F'<sup>lac</sup> and P. pseudotuberculosis F<sup>-</sup>, we found that a certain phage lysed F<sup>-</sup> but not F'<sup>lac</sup> strains. Although this phage, designated H by us, is currently used to aid in the identification of Pasteurella pestis, its host range at 37 C includes Escherichia coli. Therefore, we were able to confirm the specificity of phage H for F<sup>-</sup> cells in E. coli K12, to characterize phage H, and to compare it with other reported female-specific phages.

## II. MATERIALS AND METHODS

### A. PHAGE

The Pasteurella phage used in this study was obtained from D.C. Cavanaugh of the Walter Reed Army Research Institute, Washington, D. C. He had isolated it from sewage obtained from the Hooper Foundation in California; it is currently used in the rapid identification of Pasteurella pestis.<sup>1</sup> The phage that he isolated appears to have properties similar to those of phage P,<sup>2</sup> which was being used at the Hooper Foundation at the time. Because there is no proof of the identity of the two phages, we arbitrarily designated the phage isolated by Cavanaugh as phage H.

Phages MS2 and T3 were obtained from stock collections in this laboratory.

### B. BACTERIAL STRAINS

P. pestis strain TRU was used to propagate phage H. E. coli K12 strain 23.10.S met<sup>-</sup> F'<sup>lac</sup> was obtained from Dr. R.C. Clowes. The origin of E. coli strain K57 was described by Kondo and Mitsuhashi.<sup>3</sup> E. coli strains AB-1518 (F'<sup>14</sup>) and AB-6 (F<sup>-</sup>his<sup>-</sup>met<sup>-</sup>gal<sup>-</sup>lac<sup>-</sup>mal<sup>-</sup>xy1<sup>-</sup>S<sup>r</sup> -) were kindly supplied by Dr. E.A. Adelberg. All other strains of bacteria were from our stock collection.

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### C. MEDIA AND CULTURAL CONDITIONS

Cultures were grown in 5 ml of Difco brain heart infusion broth (BHI) in test tubes (18 x 150 mm) incubated in a slanted position on a reciprocal shaker (5-cm stroke, 100 excursions per minute) at 37 C. For phage assays, approximately  $10^8$  cells in the late logarithmic phase were used to prepare lawns.

TY medium, composed of 10 g Bacto-tryptone, 5 g Difco yeast extract, 10 g NaCl, and 2 mg  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$  per liter and adjusted to pH 7.4, was used to propagate phage. Hard agar plates contained 25 ml of either Difco blood agar base (BAB) or the PA medium of Thorne.<sup>4</sup> Soft agar contained 0.7% agar in either Difco heart infusion broth (HIB) or PA medium.

### D. PROPAGATION OF PHAGE

Large batches of phage were produced on the appropriate host growing in shaken flasks of TY broth at 37 C. The phage was usually added at a multiplicity of infection (MOI) of 0.1 after 2 hours of growth, and incubation was continued until lysis ensued. Smaller batches of phage were produced by harvesting in 1% peptone the soft agar layer of plates showing confluent lysis. Usually cellular debris was removed by centrifugation and filtration through ultrafine fritted glass filters; occasionally lysates and plate harvests were sterilized with chloroform. Neither filtration nor chloroform had any significant effect on the phage.

### E. ASSAY OF PHAGE

We used the agar layer technique<sup>5</sup> to titrate phage suspensions and to determine plaque morphology. At first we used BAB plates overlaid with HIB soft agar, but phage H plaques were so large that titrations were inaccurate. Smaller and more distinctive plaques were obtained on PA agar, which made counts much more reliable. The standard diluent for phage suspensions was HIB mixed 1:1 with 0.033 M potassium phosphate buffer, pH 7.4. Plates were incubated at 37 C.

## III. RESULTS

### A. CHARACTERISTICS OF PHAGE H

On BAB agar, phage H formed large clear plaques with large turbid halos when plated on *P. pestis* TRU. With prolonged incubation, the plaques continued to enlarge until the entire plate might be cleared. This was not due to the release of a lytic substance, because viable phage could be

recovered from any area of the plaque. We purified the phage by three successive single-plaque isolations, always picking from the large clear center. A stock preparation was propagated on P. pestis TRU in BHI broth at 37 C. The filtered lysate had a titer of  $4 \times 10^{10}$  plaque-forming units (pfu) per ml and was stored at 5 C.

On PA agar, both the clear areas and the turbid halos were smaller, and these plaques did not increase as much in size with prolonged incubation. When plated on E. coli K12 strain AB-6 on PA agar, phage H formed distinctive target-like plaques. Therefore, this strain was used routinely for assaying the phage.

A one-step growth curve of phage H was run according to the procedure of Ellis and Delbruck,<sup>6</sup> using E. coli AB-6 as the host. The results presented in Figure 1 indicate that the latent period was 10 to 12 minutes and the burst size was approximately 100.

Phage H is markedly stable even at high dilution. Its nucleic acid is of the DNA type and its morphological structure consists of small, spherical heads and very short tails.

#### B. HOST RANGE OF PHAGE H

When spotted on lawns at 37 C, phage H attacked all the strains of P. pestis and roughly half the strains of P. pseudotuberculosis tested. In addition, it attacked all  $F^-$  strains of E. coli tested except strain 58-161 $F^-$  but none of the  $F^+$ ,  $F'$ , or Hfr strains. The activity of phage H was not inhibited by the presence of  $\lambda$  prophage, col K3, or several R factors in  $F^-$  cultures. Although col I inhibited MS2 activity in one Hfr and one  $F^+$  strain, it did not render these cultures sensitive to H phage.

Phage H did not attack the following bacterial strains: Salmonella typhimurium, Salmonella enteritidis, Shigella dysenteriae, Proteus morganii, Pseudomonas aeruginosa, Enterobacter aerogenes, Arizona arizona, Klebsiella pneumoniae, and Erwinia carotovora. Some preparations at high concentrations attacked Serratia marcescens.

#### C. SPECIFICITY OF PHAGE H FOR $F^-$ STRAINS OF E. COLI

The female specificity of phage H was verified by comparing the efficiency of plating (EOP) of phage H and phage MS2 on strains of E. coli of different sexual types. As shown in Table 1, the EOP of MS2 was 1 compared with  $<10^{-9}$  for H phage on strains carrying the F factor as  $F^+$ ,  $F'$ , or Hfr. On  $F^-$  strains, the EOP was  $<10^{-10}$  for MS2 and 1 to  $10^{-1}$  for phage H with one exception. This again was on strain 58-161  $F^-$ , which was also insensitive to T3 according to Schell et al.<sup>7</sup> They proposed that this strain contained a defective F that had retained the gene controlling restriction but had lost those controlling other recognizable properties

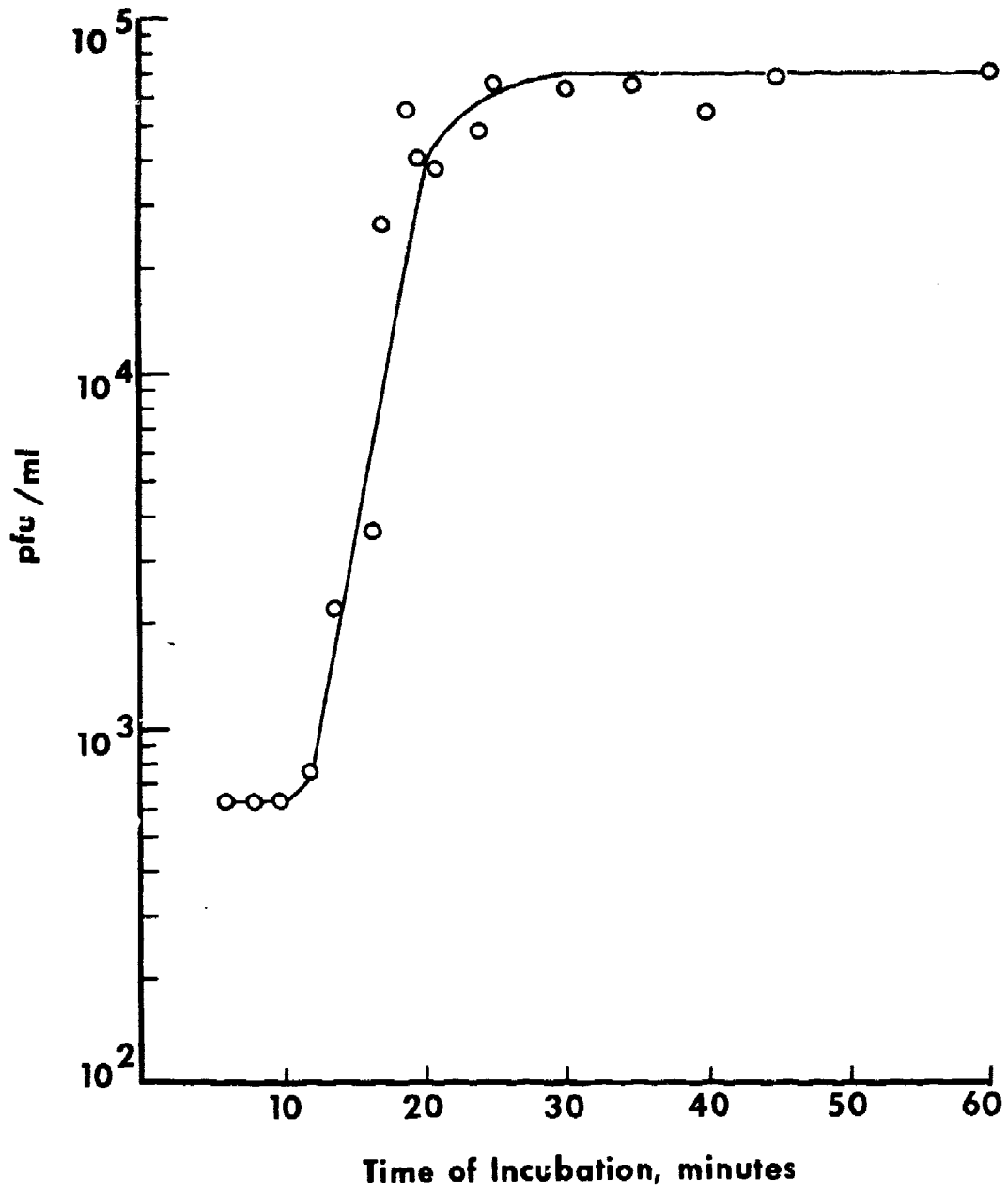


FIGURE 1. Single-Step Growth Curve of Phage H on *E. coli* AB-6.

of F. Meynell and Datta<sup>2</sup> showed that cultures of 58-161 F<sup>-</sup> contained a small proportion of MS2-sensitive cells (determined by titer increase) and interpreted their results to mean the F factor was still present but in a repressed state.

TABLE 1. INFLUENCE OF THE F FACTOR IN STRAINS OF E. COLI ON THE EFFICIENCY OF PLATING OF PHAGE H

Strain	Sexual Type	Efficiency of Plating <sup>a/</sup>	
		H	MS2
AB-6	F <sup>-</sup>	1	<10 <sup>-10</sup>
AB-359	F <sup>-</sup>	10 <sup>-1</sup>	<10 <sup>-10</sup>
C600 ( $\lambda^+$ )	F <sup>-</sup>	10 <sup>-1</sup>	<10 <sup>-10</sup>
58-161	F <sup>-</sup>	10 <sup>-9</sup>	<10 <sup>-10</sup>
58-161	F <sup>+</sup>	<10 <sup>-9</sup>	1
W-1485	F <sup>+</sup>	<10 <sup>-9</sup>	1
W-4520 (F'8)	F'	<10 <sup>-9</sup>	1
W-3747 (F'13)	F'	<10 <sup>-9</sup>	1
AB-1518 (F'14)	F'	<10 <sup>-9</sup>	10 <sup>-1</sup>
23.10 S (F'lac)	F'	<10 <sup>-9</sup>	1
Hfr C ( $\lambda^+$ )	Hfr	<10 <sup>-9</sup>	1
P4 X 6 ( $\lambda^+$ )	Hfr	<10 <sup>-9</sup>	1

- a. The titer of phage H-TRU on E. coli AB-6 was 10<sup>10</sup> pfu/ml and was assigned an EOP of 1. The titer of MS2 on Hfr C was 10<sup>11</sup> pfu/ml and was assigned an EOP of 1.

The lytic activity of phage H on broth cultures of E. coli strains of different sexual type confirmed the specificity to F<sup>-</sup> cells (Fig. 2). The phage lysed the F<sup>-</sup> strain rapidly and completely but not the F<sup>+</sup>, F', or Hfr strains even after 24 hours of incubation. Also, the phage titer increased 4 logs on the F<sup>-</sup> strain but did not increase on the other strains.

#### D. ADSORPTION OF PHAGE H

Mixtures of log-phase cells of E. coli of different sexual types and phage at an MOI of 0.1 were incubated at 35 C. Samples were removed at various time intervals, the cells were sedimented by centrifugation, and the supernatant fluid was assayed for remaining phage (Fig. 3). There was little or no adsorption to strains Hfr C, W-1485 F<sup>+</sup>, or K57 F'<sub>Cm</sub>; however, there was rapid adsorption to the F<sup>-</sup> strains AB-6 and B followed by a rapid increase of free phage after 10 minutes.

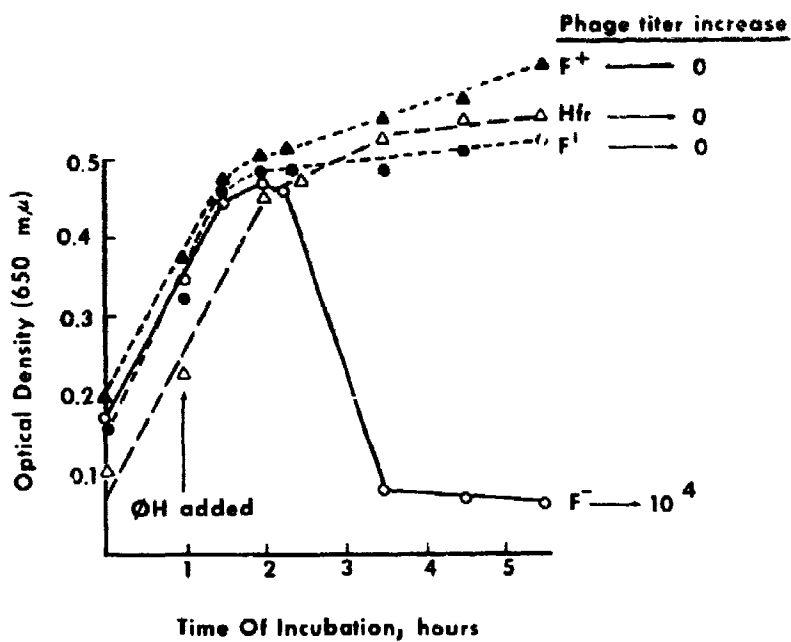


FIGURE 2. Influence of the F Factor in Strains of *E. coli* on the Lysis of Broth Cultures by Phage H.

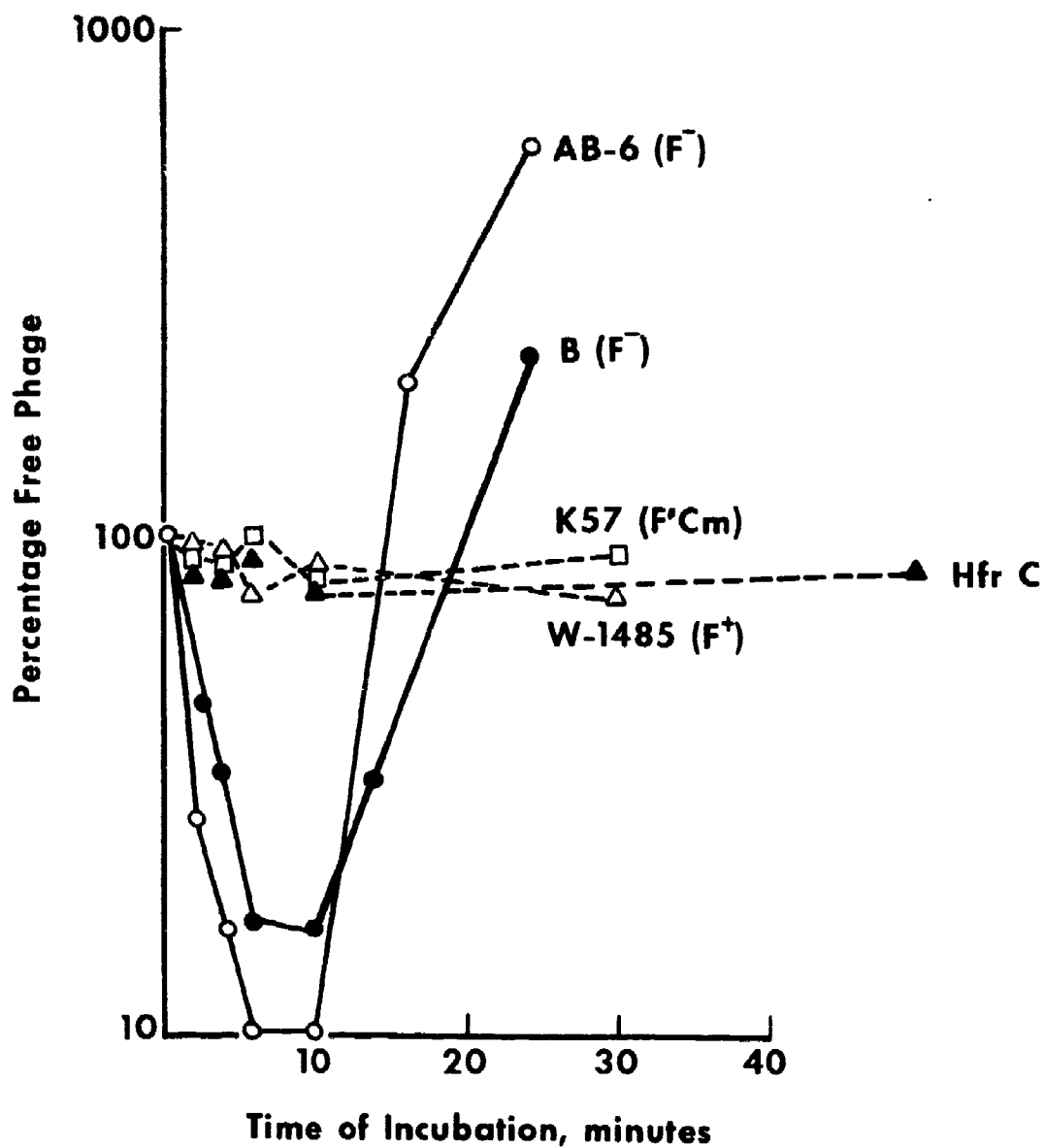


FIGURE 3. Adsorption of Phage H to Strains of *E. coli* of Different Sexual Types.

#### E. COMPARISON OF PHAGE H WITH OTHER FEMALE-SPECIFIC PHAGES

Six E. coli phages and one Salmonella typhimurium phage have been reported to be specific for F<sup>-</sup> strains. The data reported for these phages are compared with the data that we have obtained on phage H (Table 2).

Phage H appeared to be different from all of the other female-specific phages. However, Hertman<sup>9</sup> had demonstrated a serological relationship between T3 and P. pestis phage Y, and we felt that additional evidence was needed to assure that T3 and H were different phages.

#### F. EVIDENCE THAT PHAGE H AND T3 ARE DIFFERENT

A comparison of T3·B (i.e., T3 grown on strain B) and T3·AB-6 revealed significant differences between the two phage preparations.

As shown in Figure 4, rapid and nearly complete adsorption of T3·B to E. coli B was followed by a large increase in phage titer. Adsorption of T3·B to strain AB-6 was barely detectable, but again there was a large increase in titer. However, there was neither adsorption nor titer increase on Hfr C. On the other hand, T3·AB-6 was adsorbed by all three strains, although the percentage adsorbed was relatively small. Nevertheless, adsorption was followed by a rapid increase in phage titer with all three strains. Presumably, propagation of T3 in strain AB-6 selected a host range mutant that can adsorb to and grow in an Hfr strain. We consider it a host range mutant because even after repropagation on E. coli B (i.e., T3·B·AB-6·B), it maintained its ability to grow in Hfr strains.

In contrast, no host range mutant was selected by growth of phage H on strain AB-6. The results in Table 3 show that the EOP of phage H propagated on either P. pestis TRU or E. coli AB-6 were nearly identical, but the EOP of T3 propagated on E. coli strains B and AB-6 differed significantly. Whereas T3·B was restricted in the F-containing strains, T3·AB-6 was not. The EOP of MS2 on these strains was included to verify the presence or absence of F.

Phage H and T3 differed also in their activity on strains of Pasteurella. At 37 C, phage H plaqued on all strains of P. pestis and certain strains of P. pseudotuberculosis, but T3 plaqued only on P. pestis TRU.

TABLE 2. COMPARISON OF PHAGE H WITH OTHER REPORTED SEX-SPECIFIC PHAGES

Phage	Literature Citation	Latent Period, minutes	Burst Size	Adsorption to $F^+$	EOP on $F^+$	Comments <sup>a/</sup>
$\phi$ I	11	b/	b/	poor	$10^{-3}$	of the T1 to T7 series of phage, only T7 behaved similarly.
W31	17	12	106 in $F^-$ 56 in $F^+$	equally good	$10^{-1}$ to $10^{-2}$	Not restricted in stable Hfr strains, nor by col E2, $\lambda$ prophage, or two $F$ factors. Restricted by col B and col I.
tau	13	120 after UV irradiation	200	equally good	b/	Temperate; lysogenizes both $F^-$ and $F^+$ strains. Not tested on Hfr or $F'$ strains.
T7	16	12	250 in $F^-$ 102 in $F^+$	equally poor	$10^{-2}$	$F$ restriction inhibited by presence of an R factor along with other expressions of $F$ .
T3	7	13	200 in $F^-$ low in $F^+$	poor	$10^{-5}$	In <i>E. coli</i> C EOP reduced to $10^{-1}$ by $F$ . Phage not modified by growth in $F'$ strains of K-12. Not restricted in 58-161 $F^-$ .
$\phi$ II	12	15 to 20	150 in $F^-$ 2 in $F^+$	equally good	$10^{-9}$	On <i>E. coli</i> B, EOP was 1 as on $F^-$ strains of K-12.
SP6	14	15	20	equally good	$10^{-6}$	Specific for <i>Salmonella</i> ; does not adsorb to <i>E. coli</i> . Kills male cells but no progeny released.
H		10 to 12	100 to 200 in $F^-$	poor	$<10^{-9}$	Attacks all strains of <i>P. pestis</i> . Not inhibited by col K-3, $\lambda$ prophage, or several R factors.

a. All the female-specific phages (plaque morphology not described for tau and SP6) produced large clear plaques with large turbid halos on  $F^-$  strains and, when present, very small plaques on strains harboring the  $F$  factor.

b. Not reported.



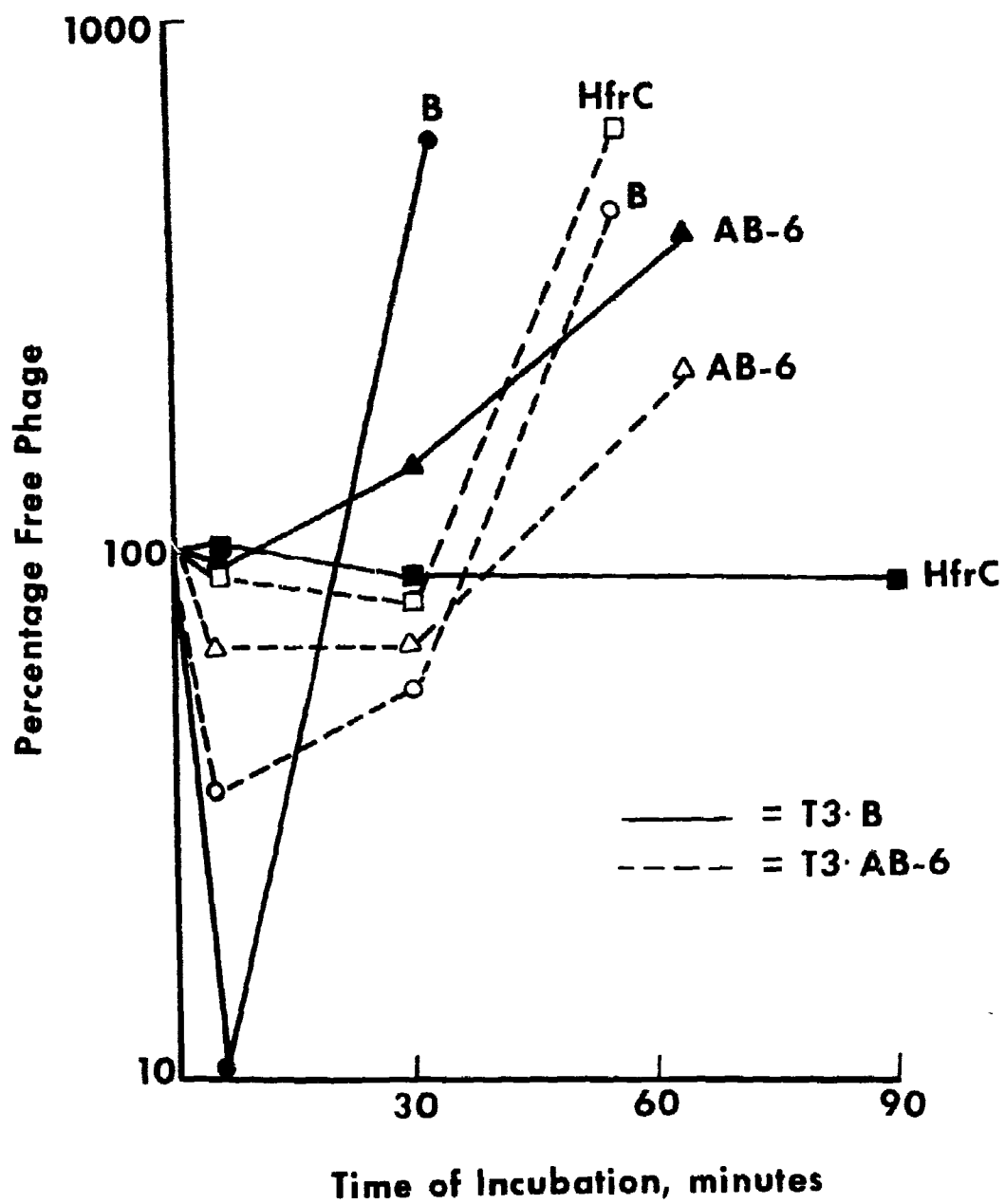


FIGURE 4. Adsorption of T3 to *E. coli* B, AB-6, and HfrC.

TABLE 3. DIFFERENTIATION BETWEEN PHAGES H AND T3 BY SELECTION OF A HOST RANGE MUTANT OF T3 ON E. COLI AB-6

Lawn	Sexual Type	Efficiency of Plating <sup>a</sup> /				MS2
		H·TRU	H·AB-6	T3·B	T3·AB-6	
AB-6	F <sup>-</sup>	1	1	1	1	<10 <sup>-10</sup>
B	F <sup>-</sup>	1	1	1	1	<10 <sup>-10</sup>
AB-359	F <sup>-</sup>	10 <sup>-1</sup>	1	10 <sup>-1</sup>	1	<10 <sup>-10</sup>
58-161	F <sup>+</sup>	<10 <sup>-9</sup>	<10 <sup>-8</sup>	<10 <sup>-9</sup>	10 <sup>-1</sup>	1
W-1485	F <sup>+</sup>	<10 <sup>-9</sup>	<10 <sup>-8</sup>	<10 <sup>-9</sup>	10 <sup>-1</sup>	1
Hfr C	Hfr	<10 <sup>-9</sup>	<10 <sup>-8</sup>	<10 <sup>-9</sup>	10 <sup>-1</sup>	1

a. EOP of H and T3 were based on titers on AB-6; EOP of MS2 was based on titer on Hfr C.

#### IV. DISCUSSION

The results reported here have shown clearly that phage H does not plaque on strains of E. coli carrying the F factor. Some of its characteristics are similar to those reported for other female-specific phages (Table 2). All except phage tau produce large clear plaques on F<sup>-</sup> strains and have a very short latent period. Tau is also exceptional in being a temperate phage, although T3 and T7 are said to give semitemperate mutants.<sup>10</sup> Only ØII approached the female specificity demonstrated by phage H based on the EOP on F<sup>+</sup> strains; however, ØII was adsorbed to F<sup>+</sup> strains equally as well as to F<sup>-</sup> strains. Hertman<sup>9</sup> discovered a serological relationship between T3 and E. pestis phage Y. Although they were indistinguishable morphologically, T3 did not form plaques on P. pestis. He concluded that phage Y has an antigenic site in common with T3 plus another site functional in the infection of P. pestis. The relationship of P. pestis phages Y and H is unknown.

Phage H was not adsorbed by F<sup>+</sup>, F', or Hfr strains of E. coli but was rapidly adsorbed by F<sup>-</sup> strains. A similar observation by Dettori, Maccacaro, and Piccinin<sup>11</sup> with Ø1 led them to postulate that the Ø1 receptor was present on both F<sup>+</sup> and F<sup>-</sup> cells but was functionally covered by F pili on F<sup>+</sup> cells. Our observation that col I inhibited F function (determined by MS2 sensitivity) but failed to render the cells sensitive to phage H did not support Dettori's hypothesis.

On the other hand, phage  $\phi$ 11, tau, and SP-6<sup>12-14</sup> were shown to be equally well adsorbed by  $F^+$  and  $F^-$  strains, indicating that the phages were inhibited at some stage after adsorption. Makura, Otsuji, and Hirota<sup>15</sup> assumed that  $F^+$  cells produced a repressor that inhibited multiplication of phage tau. Makela, Makela, and Soikkeli<sup>16</sup> suggested that a product of  $F$  might act as a repressor of T7, and Schell et al.<sup>7</sup> also postulated that restriction of T3 may be controlled by a gene in the  $F$  episome. However, Watanabe and Okada<sup>17</sup> stated that suppression of phage W31 by  $F^+$  strains but not by Hfr strains could not be due to the usual repressor of  $F$  but might be due to a metabolic competition in replication between  $F$  and W31. Siccardi<sup>18</sup> found that T1, T3, and  $\lambda$ vir were all restricted by R factors that restricted W31 and BF 23 even in  $r^-m^-$  mutants of E. coli K12.

Our data show that T3, like phage H, is not adsorbed to strain Hfr C. T3, unlike phage H, is very poorly adsorbed to the  $F^-$  strain AB-6, although it propagates well on it and plates efficiently on  $F$ -containing strains after growth on AB-6. Our data suggest that this is a host range mutation and not a host-induced modification. Other workers have also reported on the absence of host-induced modification with T3.<sup>7,19,20</sup> Bannister and Glover<sup>21</sup> indicated that the reduction in the EOP and in plaque size of so-called female-specific phages (T3, W31, and  $\phi$ 1) by  $F$  is not the result of host modification but is more likely due to one or more of the following: (i) reduction in burst size, (ii) inefficient adsorption, or (iii) slightly impaired transmission.

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13. ABSTRACT Phage H, thought to be specific for <u>Pasteurella pestis</u> , was shown to plate efficiently on F <sup>-</sup> strains of <u>Escherichia coli</u> but not on F <sup>+</sup> , P <sup>+</sup> , or Hfr strains. The phage was adsorbed rapidly to F <sup>-</sup> strains but was not adsorbed to strains carrying F. Comparison with seven other reported female-specific phages showed that, although phage H was similar to the other phages in some characteristics, the exceptionally low efficiency of plating (<10 <sup>-9</sup> ) on F-containing cells makes phage H a particularly useful female-specific phage. ←		
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